Claims

(1) A method for quantitatively determining LDL cholesterol in a biological sample, which comprises performing the reaction of cholesterol in the presence of:

a) a biological sample,

b) cholesterol esterase and cholesterol oxidase or cholesterol dehydrogenase (hereinafter collectively referred to as CH enzymes), and

c) a reagent enabling the CH enzymes of b) to act only on LDL cholesterol, and

measuring the amount of the hydrogen peroxide or reduced coenzyme formed by the reaction to quantitatively determine the concentration of LDL cholesterol.

(2) The method according to claim (1), wherein the reagent enabling CH enzymes to act only on LDL cholesterol is a reagent containing at least a polyoxyethylene derivative and a polyoxyethylene-polyoxypropylene copolymer.

(3) The method according to claim (2), wherein the polyoxyethylene derivative is a polyoxyethylene alkyl ether or a polyoxyethylene alkylaryl ether.

(4) The method according to claim (2) or (3), wherein the polyoxyethylene-polyoxypropylene copolymer is a surfactant represented by general formula (I):

$$HO-(C_2H_4O)_a-(C_3H_6O)_b-(C_2H_4O)_c-H \qquad (I)$$

(wherein a, b and c, which may be the same or different, each represents an integer of 1 to 200).

(5) A method for the continuous fractional determination of HDL cholesterol and LDL cholesterol in a biological sample, which comprises

subjecting cholesterol to the first reaction in the presence of:

a) a biological sample,

b) CH enzymes, and

c) a reagent enabling the CH enzymes of b) to act only on HDL cholesterol, and

measuring the amount of the hydrogen peroxide or reduced coenzyme formed by the first reaction to quantitatively determine the concentration of HDL cholesterol,

then adding

d) a reagent enabling the CH enzymes of b) to act only on LDL cholesterol,

subjecting cholesterol to the second reaction, and

measuring the amount of the hydrogen peroxide or reduced coenzyme formed by the second reaction to quantitatively determine the concentration of LDL cholesterol.

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(6) A method for the continuous fractional determination of HDL cholesterol and LDL cholesterol in a biological sample, which comprises

subjecting cholesterol to the first reaction in the presence of:

a) a biological sample,

b) CH enzymes, and

c) a reagent enabling the CH enzymes of b) to act only on HDL cholesterol, and

measuring the amount of the hydrogen peroxide or reduced coenzyme formed by the first reaction to quantitatively determine the concentration of HDL cholesterol,

then adding

d) CH enzymes, and

e) a reagent enabling the CH enzymes of d) to act only on LDL cholesterol,

subjecting cholesterol to the second reaction, and

measuring the amount of the hydrogen peroxide or reduced coenzyme formed by the second reaction to quantitatively determine the concentration of LDL cholesterol.

(7) The method according to claim (5) or (6), wherein the reagent enabling CH enzymes to act only on LDL cholesterol is a reagent containing ~~at least~~ a polyoxyethylene derivative and a polyoxyethylene-polyoxypropylene copolymer.

(8) The method according to claim (7), wherein the polyoxyethylene derivative is a polyoxyethylene alkyl ether or a polyoxyethylene alkylaryl ether.

(9) The method according to claim (7) or (8), wherein the polyoxyethylene-polyoxypropylene copolymer is a surfactant represented by general formula (I):

$$HO-(C_2H_4O)_a-(C_3H_6O)_b-(C_2H_4O)_c-H \qquad (I)$$

(wherein a, b and c, which may be the same or different, each represents an integer of 1 to 200).

(10) A method for the continuous fractional determination of HDL cholesterol and total cholesterol in a biological sample, which comprises

subjecting cholesterol to the first reaction in the presence of:

a) a biological sample,

b) CH enzymes, and

c) a reagent enabling CH enzymes of b) to act only on HDL cholesterol, and

measuring the amount of the hydrogen peroxide or reduced coenzyme formed by the first reaction to quantitatively determine the concentration of HDL cholesterol,

then adding

d) a reagent enabling the CH enzymes of b) to act on cholesterol in all lipoproteins,

subjecting cholesterol to the second reaction, and

measuring the amount of the hydrogen peroxide or reduced coenzyme formed by the second reaction to quantitatively determine the concentration of total cholesterol.

(11) A method for the continuous fractional determination of HDL cholesterol and total cholesterol in a biological sample, which comprises

subjecting cholesterol to the first reaction in the presence of:

a) a biological sample,

b) CH enzymes, and

c) a reagent enabling the CH enzymes of b) to act only on HDL cholesterol, and

measuring the amount of the hydrogen peroxide or reduced coenzyme formed by the first reaction to quantitatively determine the concentration of HDL cholesterol,

then adding

d) CH enzymes, and

e) a reagent enabling the CH enzymes of d) to act on cholesterol in all lipoproteins,

subjecting cholesterol to the second reaction, and

measuring the amount of the hydrogen peroxide or reduced coenzyme formed by the second reaction to quantitatively determine the total cholesterol.

(12) The method according to any one of claims (5) through (11), wherein the reagent enabling CH enzymes to act only on cholesterol in HDL is a reagent for aggregating lipoproteins other than HDL.

(13) The method according to claim (12), wherein the reagent for aggregating lipoproteins other than HDL further contains a nonionic surfactant that does not solubilize the aggregated lipoproteins.

(14) The method according to claim (12) or (13), wherein the reagent for aggregating lipoproteins other than HDL is a reagent comprising heparin or a salt thereof, phosphotungstic acid or a salt thereof, dextran sulfuric acid or a salt thereof, polyethylene glycol, sulfonated cyclodextrin or a salt thereof, sulfonated oligosaccharide or a salt thereof, or a mixture thereof and a divalent metal salt.

(15) The method according to claim (6) or (11), wherein the CH enzymes used in the first reaction of cholesterol are chemically modified enzymes and the CH enzymes used in the second reaction of cholesterol are enzymes that are not chemically modified.

(16) The method according to any one of claims (10) through (15), wherein the reagent enabling the CH enzymes to

act on cholesterol in all lipoproteins is a reagent containing a lipoprotein solubilizing surfactant.

(17) A reagent for determining LDL cholesterol comprising CH enzymes and a reagent enabling the CH enzymes to act only on LDL cholesterol.

(18) The reagent for determining LDL cholesterol according to claim (17), wherein the reagent enabling the CH enzymes to act only on LDL cholesterol is a reagent containing at least a polyoxyethylene derivative and a polyoxyethylene-polyoxypropylene copolymer.

(19) The reagent according to claim (18), wherein the polyoxyethylene derivative is a polyoxyethylene alkylaryl ether.

(20) The reagent according to claim (18) or (19), wherein the polyoxyethylene-polyoxypropylene copolymer is a surfactant represented by general formula (I):

$$HO-(C_2H_4O)_a-(C_3H_6O)_b-(C_2H_4O)_c-H \qquad (I)$$

(wherein a, b and c, which may be the same or different, each represents an integer of 1 to 200).

(21) A reagent kit for the fractional determination of HDL cholesterol and LDL cholesterol comprising a first reagent and a second reagent, said first reagent comprising a reagent for aggregating lipoproteins other than HDL lipoprotein and a reagent containing CH enzymes, and said second reagent comprising a reagent enabling CH enzymes to act only on LDL cholesterol.

(22) The reagent kit according to claim (21), wherein the reagent enabling CH enzymes to act only on LDL cholesterol is a reagent containing a polyoxyethylene derivative and a polyoxyethylene-polyoxypropylene copolymer.

(23) The reagent kit according to claim (21), wherein the polyoxyethylene derivative is a polyoxyethylene alkylaryl ether.

(24) The reagent kit according to claim (21) or (22), wherein the polyoxyethylene-polyoxypropylene copolymer is a surfactant represented by general formula (I):

$$HO-(C_2H_4O)_a-(C_3H_6O)_b-(C_2H_4O)_c-H \qquad (I)$$

(wherein a, b and c, which may be the same or different, each represents an integer of 1 to 200).

(25) A reagent kit for the fractional determination of HDL cholesterol and total cholesterol comprising a first reagent and a second reagent, said first reagent comprising a reagent for aggregating lipoproteins other than HDL lipoprotein and a reagent containing CH enzymes, and said second reagent comprising a reagent enabling CH enzymes to act on cholesterol in all lipoproteins.

(26) The reagent kit according to claim (25), wherein the reagent enabling CH enzymes to act on cholesterol in all lipoproteins further contains a lipoprotein solubilizing surfactant.

(27) The reagent kit according to any one of claims (21) through (26), wherein the reagent for aggregating lipoproteins other than HDL lipoprotein is a reagent comprising heparin or a salt thereof, phosphotungstic acid or a salt thereof, dextran sulfuric acid or a salt thereof, polyethylene glycol, sulfonated cyclodextrin or a salt thereof, sulfonated oligosaccharide or a salt thereof, or a mixture thereof and a divalent metal salt.